The Importance of Taurine and n-3 Fatty Acids in Cobia, *Rachycentron canadum*, Nutrition

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Abstract: In the wild, fish can adapt to their diet by down regulating or eliminating biosynthetic pathways for nutrients found in abundance in their prey. Upon domestication for aquaculture these fish may now have unique dietary requirements which must be met through supplementation. To examine this hypothesis and aid in determining potential minimum requirement levels for aquaculture production, we have examined the synthetic capacity for taurine and polyunsatured fatty acids in cobia, *Rachycentron canadum*, through molecular methods and growth trials, respectively.

Taurine, a non-protein amino acid, is found in high concentrations in the natural diet of cobia and plays a variety of physiological roles. The taurine biosynthesis pathway in vertebrates is well known; however it appears to be nonfunctional in some terrestrial (Felidae) and marine (Rachycentridae) carnivores, thereby necessitating dietary supplementation. Taurine is not found in plant protein sources used for replacing fishmeal in aquaculture feeds, an increasingly important priority.

We examined the effects of graded levels of taurine addition (0%, 0.5%, 1.5%, 5.0%) to plant protein and fishmeal based diets formulated and manufactured by the USDA-ARS. At the conclusion of a feeding trial, RNA was extracted from liver, muscle, and brain tissue for quantitative-RT-PCR analysis of the genes involved in taurine synthesis. Cysteine dioxygenase (CDO), cysteamine dioxygenase (ADO), and taurine transporter (TauT) activity and expression levels were examined and no differences in transcript abundance was detected within the tissues between the dietary taurine levels. Increasing dietary taurine resulted in increased tissue taurine concentrations.

To examine the effects of alternative sources completely replacing fish oil, two replacements were examined as the lipid sources in a fishmeal free, plant based feed (USDA-ARS) and compared to a fish oil version of the diet. A thraustochytrid meal plus soybean oil (TM+SOY) and a canola oil with exogenous docosahexaenoic (DHA) and arachidonic (ARA) acids (CO+EFA) were utilized. At the conclusion of an eight week growth trial, whole body and fillet fatty acid profiles were examined.

The TM+SOY diet worked equivalently to the USDA-ARS control diet, however the CO+EFA diet resulted in significantly lower growth and survival as well as an increased feed conversion ratio. Whole body fatty acid profiles revealed a significant reduction in total essential fatty acid (EFA) concentration in the CO+EFA fed fish, indicating the supplemented levels of DHA and ARA were insufficient to meet requirements. Although all three diets were sufficient in the precursors for EFA synthesis, this reduction in growth, survival, and whole body EFA concentration suggests cobia have limited synthetic capacity for DHA and ARA. Both alternative lipid diets were devoid of eicosapentanoic acid (EPA) supplementation, which does not appear to be essential for cobia as the TM+SOY diet performed equivalently to the fish oil control.

Key words: Cobia, Taurine, Docosahexaenoic Acid, Fishmeal and Fish Oil Replacement

World fish supply has grown on average 3.2 percent per year over the last five decades, with much of the growth occurring in various aquaculture sectors (FAO, 2012). With worldwide capture fisheries holding relatively static over the last few decades, and expected to remain at current levels, aquaculture must continue to expand rapidly to meet the protein demands of the ever increasing world population. Traditionally, fishmeal and fish oil acquired through reduction of wild capture fisheries have served as major protein and lipid sources, respectively, for compounded fish feeds utilized for many species in culture. In order for aquaculture to continue to grow at current and desired rates, the inclusion levels of these ingredients must be continually reduced or eliminated, as their abundance is finite. The reduction of fishmeal and fish oil use as well as alternate protein and lipid sources have been studied extensively in many species and has been established as a priority for research efforts and the aquaculture industry as a whole (Rust *et al.*, 2011).

Reductions in productivity including decreased growth rates, increased feed conversion ratios, low feed intake, and negative health impacts have been observed in many species when reducing fishmeal below species specific threshold levels (Glencross *et al.*, 2011; Kader *et al.*, 2012; Luo *et al.*, 2006; Thiessen *et al.*, 2004; Wacyk *et al.*, 2012; Xie *et al.*, 1998). This is often related to deficiencies in amino acids, fatty acids, vitamins, or minerals that are not present in sufficient quantities in sources utilized as fishmeal replacements. This is especially true when utilizing plant proteins (wheat flour, corn gluten, soybean meal, barley, etc) as plants have substantially different amino acid profiles when compared to fishmeals or other meat based protein meals. Many of these deficiencies have been identified (lysine, methionine, threonine) and can be corrected for by simple crystalline amino acid addition to feed formulations. One challenge brought about by reducing fishmeal in favor of plant proteins is in identifying deficiencies on a species specific basis and then determining minimal and optimal supplementation levels. Similarly, when reducing or replacing fish oil in favor of alternative lipid sources, some specific fatty acids critical to growth and health are lacking in terrestrial oil sources when compared to fish oil.

Taurine, a non-protein amino acid, is found in high concentrations in fishmeal, wild prey items, and meat based meals but is devoid from plant protein sources. Taurine plays multiple important physiological roles as an osmolyte, antioxidant, photoreceptor protectant, and as a bile salt conjugate (Schuller-Levis and Park, 2003). Multiple studies have shown advantages to supplementing taurine when reducing fishmeal in a variety of species (Chatzifotis *et al.*, 2008; Kim *et al.*, 2005, 2003; Lunger *et al.*, 2007; Pinto *et al.*, 2010).

Long chain polyunsaturated fatty acids, such as docosahexaenoic (DHA) and arachidonic (ARA) acids are also not typically observed in terrestrial oils, and the ability of teleost species to convert precursors into these important fatty acids is typically low and highly variable, rendering them essential for many marine species. As with taurine and several other compounds, these fatty acids in particular must be supplemented to feeds utilizing alternative protein or lipid sources.

Cobia, *Rachycentron canadum*, is a fast growing marine carnivore whose natural diet is high in fish and crabs (Arendt *et al.*, 2001; Franks *et al.*, 1996) and is therefore likely sufficient in both taurine and the essential fatty acids DHA and ARA. Cobia is a promising species for intensive aquaculture as they can be spawned and maintained in captivity, are highly fecund serial spawners, and have high quality flesh suitable for multiple consumer types (Holt *et al.*, 2007). A great deal of research has focused on cobia nutrition and reducing and replacing both fishmeal and fish oil with alternative sources to develop sustainable feeds (Lunger *et al.*, 2006; Salze *et al.*, 2010; Trushenski *et al.*, 2012; Watson *et al.*, 2012).

Here, several studies were carried out with juvenile cobia to determine the potential synthetic capacity for taurine present in various tissues and to evaluate two fishmeal and fish oil free diets.

Materials and Methods

To examine the effects of dietary taurine on growth and performance, as well as the taurine synthetic capacity of juvenile cobia, four diets

Table 1. Dietary formulations for the diets with graded levels of taurine. From Watson *et al.* 2014.

Ingredient (g kg^{-1})	FM1	FM2	FM3	FM4
Menhaden Fish Meal	345	345	345	345
Corn Protein concentrate	44.3	44.3	44.3	44.3
Poultry by-product meal	118	118	118	118
Wheat Flour	242.7	237.7	227.7	192.7
Soybean meal, solvent extracted	90	90	90	90
Blood meal, spray dehydrated	39	39	39	39
Menhaden Fish Oil	90	90	90	90
Vitamin Pre-mix[1]	20	20	20	20
Choline CL	6	6	6	6
Stay-C	2	2	2	2
Trace mineral pre-mix[2]	1	1	1	1
Mycozorb	2	2	2	2
Taurine	0	5	15	50

[1] Contributed per kg diet; vitamin A, 9650 IU; vitamin D, 6.6 IU; vitamin E, 132 IU; menadione sodium bisulfite, 4.7 mg; thiamine mononitrate, 9.1 mg; riboflavin, 9.6 mg; pyridoxine hydrochloride, 13.7 mg; pantothenate, DL-calcium, 101.1 mg; cyanocobalamine, 0.03 mg; nictonic acid, 21.8 mg; biotin, 0.33 mg; folic acid, 2.5 mg.
[2] Contributed in mg kg^{-1} of diet; zinc 37; manganese, 10; iodine, 5; copper, 1.

were formulated to contain 0.0%, 0.5%, 1.5%, and 5.0% supplemental taurine to a fishmeal based diet (Table 1). Juvenile cobia (~125g) were housed in a recirculating aquaculture system at the Institute of Marine and Environmental Technology in Baltimore, Maryland, USA and fed for 8 weeks. At the conclusion of the eight week feeding trial, growth and feed conversion ratios were calculated, and tissues (muscle, liver, and brain) were preserved in RNA later for quantitative-PCR analysis of several genes involved in taurine synthesis. Plasma, liver, and muscle tissues were also taken for taurine content analysis. Full materials and methods are available in Watson *et al.* (2014). A parallel study was conducted with a fishmeal free, plant protein based formulation also containing 0.0%, 0.5%, 1.5%, and 5.0% supplemental taurine and similar analyses were conducted at the conclusion of the trial.

To examine the effects of fish oil replacement in a fishmeal free, plant protein based diet similar methods were employed. Two experimental diets were formulated and manufactured by the USDA-ARS (Table 2). One experimental diet utilized

canola oil plus exogenous DHA (~2.85 g kg^{-1}) and ARA (~0.32 g kg^{-1}) (CO+EFA diet) while the other experimental diet utilized a thraustochytrid meal plus soybean oil (TM+SOY) as the lipid source. The control diet consisted of the same plant protein base as the experimental diets, but utilized fish oil as the lipid source. An eight week growth trial was conducted beginning with ~130g juvenile cobia and at the conclusion growth and feed conversion ratio were calculated, and fatty acid profiles of fillets were determined through gas chromatography (GC). Full materials and methods are available in Watson *et al.*, 2013a.

Results

Full results of both studies are available in the cited references, only a brief synopsis is presented here. Taurine supplementation to a fishmeal (~35%) diet did not have significant effects on growth rate, protein efficiency ratio, total bile salt concentration, or feed conversion ratio (Table 3). Transcript abundances of CDO, ADO, and TauT were also not affected by dietary taurine input level in any of the tissues examined (Fig. 1), however the rate limiting step of the reaction, cysteinesulfinate decarboxylase (CSD) was not detected through the q-PCR methods utilized. Tissue taurine levels for plasma, liver, and muscle did increase with increasing dietary taurine.

In contrast, taurine supplementation to the plant protein based diet did have significant effects on growth rate and feed conversion ratio although overall growth was significantly lower in all treatments of the plant protein formulation when compared to the fishmeal based series (Table 4). Similarly to the fishmeal series, transcript abundances for CDO, ADO, and TauT were unaffected by dietary taurine input (Fig. 1).

In the fish oil replacement study, the TM+SOY diet performed similarly to the ARS control fish oil diet while the CO+EFA diet significantly underperformed in regards to growth rate, feed conversion ratio, and survival (Table 5; Watson *et al.*, 2013a). The different lipid sources utilized in the diets did affect whole body and fillet lipid profiles, with direct similarity to the profiles of the diets (Fig. 2; Watson *et al.*, 2013a). The alternate lipid

Table 2. Diet formulations and fatty acid compositions of the diets used for fish oil replacement. From Watson *et al.,* 2013a.

	Diet		
Ingredient (g kg^{-1})	TM+SOY	CAN+EFA	ARS Control
Soy Protein Concentrate	269.3	269.3	269.3
Corn Gluten	211	211	211
Wheat Flour	226.5	226.5	226.5
Soybean Meal			
Solvent Extracted	121	121	121
Menhaden Oil	0	0	84
Soybean Oil	5	0	0
Algamac 3050	79	0	0
Canola Oil + DHA + ARA	0	84	0
Dicalcium Phosphate	23.7	23.7	23.7
Vitamin Pre-mix[1]	10	10	10
Lysine-HCL	15.5	15.5	15.5
Choline CL	6	6	6
Trace Mineral Pre-mix[2]	1	1	1
Magnesium Oxide	0.5	0.5	0.5
Stay-C	3	3	3
DL-Methionine	5.8	5.8	5.8
Threonine	2.1	2.1	2.1
Potassium Chloride	5.6	5.6	5.6
Taurine	15	15	15
Fatty Acid (g 100g^{-1})[1]			
12:0	0.15	0.34	0.10
14:0	4.33	1.21	4.88
16:0	17.17	7.73	22.89
17:0	0.16	0.15	0.47
18:0	2.77	3.49	5.31
20:0	0.28	0.82	0.30
22:0	0.24	0.38	0.14
SFA[3]	25.10	14.12	34.08
16:1*n*-7	0.23	0.46	5.44
18:1*n*-7	0.87	2.58	2.46
18:1*n*-9+6	13.86	48.28	19.53
20:1*n*-15+cis-8	0.06	0.03	0.22
20:1*n*-9	0.14	0.76	1.21
24:1*n*-9	0.0	0.13	0.32
MUFA[4]	15.15	52.24	29.18
16:3*n*-4	0.05	0.06	0.57
16:4*n*-1	0.04	0.06	0.79
18:2*n*-6	33.27	22.86	12.74
18:3*n*-3	3.94	6.25	1.19
20:2*n*-6	0.04	0.13	0.18
20:4*n*-6	0.70	0.32	0.84
20:5*n*-3	0.42	0.27	8.90
22:5*n*-6	6.09	0.66	0.31
22:5*n*-3	0.16	0.16	1.51
22:6*n*-3	14.93	2.85	8.57
PUFA[5]	59.75	33.64	36.75
n-3[6]	19.45	9.53	20.17
n-6[7]	40.10	23.97	14.07
n-3:*n*-6	0.49	0.40	1.43

[1] Contributed per kg diet; vitamin A, 13510 IU; vitamin D, 9.2 IU; vitamin E, 184.4 IU; menadione sodium bisulfite, 6.6 mg; thiamine mononitrate, 12.7 mg; riboflavin, 13.4 mg; pyridoxine hydrochloride, 19.2 mg; pantothenate, DL-calcium, 141.5 mg; cyanocobalamine, 0.04 mg; nictonic acid, 30.5 mg; biotin, 0.46 mg; folic acid, 3.5 mg.
[2] Contributed in mg kg^{-1} of diet; zinc 37; manganese, 10; iodine, 5; copper, 1.
[3] Saturated fatty acids = sum of all fatty acids without double bonds.
[4] Monounsaturated fatty acids = sum of all fatty acids with a single double bond.
[5] Polyunsaturated fatty acids = sum of all fatty acids with two or more double bonds.
[6] Sum of all n-3 fatty acids.
[7] Sum of all n-6 fatty acids

Table 3. Performance characteristics from the graded taurinein a fishmeal diet growth trial. Within a row, different letters indicate significant differences ($P<0.05$). From Watson *et al.* 2014.

Diet	FM1	FM2	FM3	FM4
Initial Weight (mean ± SD)	127.44 ± 13.31	122.70 ± 14.97	130.64 ± 12.37	127.36 ± 14.52
Survival (%)	100	100	100	100
Weight Gain (g fish⁻¹)[1]	227.83 ± 18.15	297.62 ± 24.20	313.47 ± 31.36	290.08 ± 33.11
FCR[2]	1.52 ± 0.10	1.42 ± 0.02	1.43 ± 0.11	1.51 ± 0.05
SGR[3]	2.1 ± 0.12	2.2 ± 0.04	2.2 ± 0.14	2.1 ± 0.09
PER[4]	1.35 ± 0.15	1.44 ± 0.04	1.26 ± 0.43	1.29 ± 0.07
HSI [5]	3.14 ± 0.14	2.99 ± 0.18	2.78 ± 0.45	2.72 ± 0.19
Total Bile Salts (mM)	28.48 ± 4.79	32.69 ± 6.04	30.02 ± 4.34	29.59 ± 2.32

[1]Weight Gain (g fish) = (final weight (avg per fish) − initial weight (avg per fish)

[2] Feed Conversion Ratio (FCR) = $(\frac{\text{food fed (g)}}{\text{weight gained (g)}})$

[3]Specific Growth Rate (SGR) = $100 * (\frac{\text{ln final weight (g)} - \text{ln initial weight(g)}}{\text{days of growth trial}})$

[4]Protein Efficiency Ratio (PER) = $(\frac{\text{weight gained (g)}}{\text{protein fed (g)}})$

[5]Hepatosomatic Index (HSI) = $100 * (\frac{\text{liver weight (g)}}{\text{body weight (g)}})$

Table 4. Performance characteristics from the graded taurine in a plant based diet growth trial (128.37 g initial weight). Within a row, values that share common superscripts are not significantly different from one another ($P >0.05$).

Diet (Taurine %)	PP1 (0.02)	PP2 (0.39)	PP3 (1.35)	PP4 (4.08)
Weight Gain (%)	23.35 ± 22.82[a]	80.57 ± 60.24[a]	130.87 ± 25.20[b]	133.82 ± 10.83[b]
FCR[1]	6.38 ± 1.49[a]	2.97 ± 1.17[b]	1.98 ± 0.19[b]	2.12 ± 0.23[b]
SGR[2]	0.57 ± 0.12[a]	1.31 ± 0.26[b]	1.47 ± 0.19[b]	1.51 ± 0.09[b]
Total Bile Salts (mM)	38.76 ± 7.40	28.37 ± 1.94	36.30 ± 5.69	28.75 ± 3.22

[1] Feed Conversion Ratio (FCR) = $(\frac{\text{food fed (g)}}{\text{weight gained (g)}})$

[2]Specific Growth Rate (SGR) = $100 * (\frac{\text{ln final weight (g)} - \text{ln initial weight(g)}}{\text{days of growth trial}})$

Table 5. Production characteristics from the eight week grow out trial with fish oil replacement sources. Data from a previous grow out with juvenile cobia on a fish meal and fish oil based, commercially produced diet are included to show overall effects of fish meal and fish oil replacement. From Watson *et al.*, 2013a.

	TM+SOY	CAN+EFA	ARS Control	Commercial Diet (2009 grow out data)[6]
Weight Gain (%)[1]	288[a]	117[b]	275[a]	218
Fillet Yield (%)[2]	25.93 ± 3.34	20.88 ± 4.34	25.71 ± 1.90	20.12 ± 0.01
FCR[3]	1.42[a]	2.98[b]	1.46[a]	1.85
Plasma Osmolality	358.25 ± 30.59[a]	311.75 ± 36.82[b]	327.58 ± 13.83[a,b]	na
PCB Content (ng g⁻¹)	8.40 ± 1.54	9.30 ± 3.41	13.2 ± 4.2	45.5 ± 3.8
Mercury Content(ng g⁻¹)	30.20 ± 3.53	48.17 ± 16.24	20.14 ± 2.96	71.37 ± 3.56
Hepatosomatic index[4]	1.83 ± 0.19[a]	2.93 ± 0.79[b]	2.66 ± 0.55[a,b]	3.15 ± 0.002
Specific Growth Rate[5]	2.48[a]	1.42[b]	2.36[a]	1.93
Survival	100%[b]	58%[a]	98%[b]	100%

[1] Weight gain=(final tank weight - initial tank weight)/ initial tank weight*100.
[2] Fillet yield=(fillet weight/body weight)*100.
[3] FCR=Feed conversion ratio= grams fed/grams gained.
[4]Hepatomsomatic index=liver weight/ body weight.
[5] SGR=specific growth rate= $((\text{lnBW}_2\text{-lnBW}_1)*(\text{days of growth trial}^{-1}))*100$.
[6] Initital Weight 120g, eight week growth trial (Watson *et al.* 2012).
Values in the same row with different superscripts are significantly different (p<0.05), no superscript indicates no significant difference within a category.
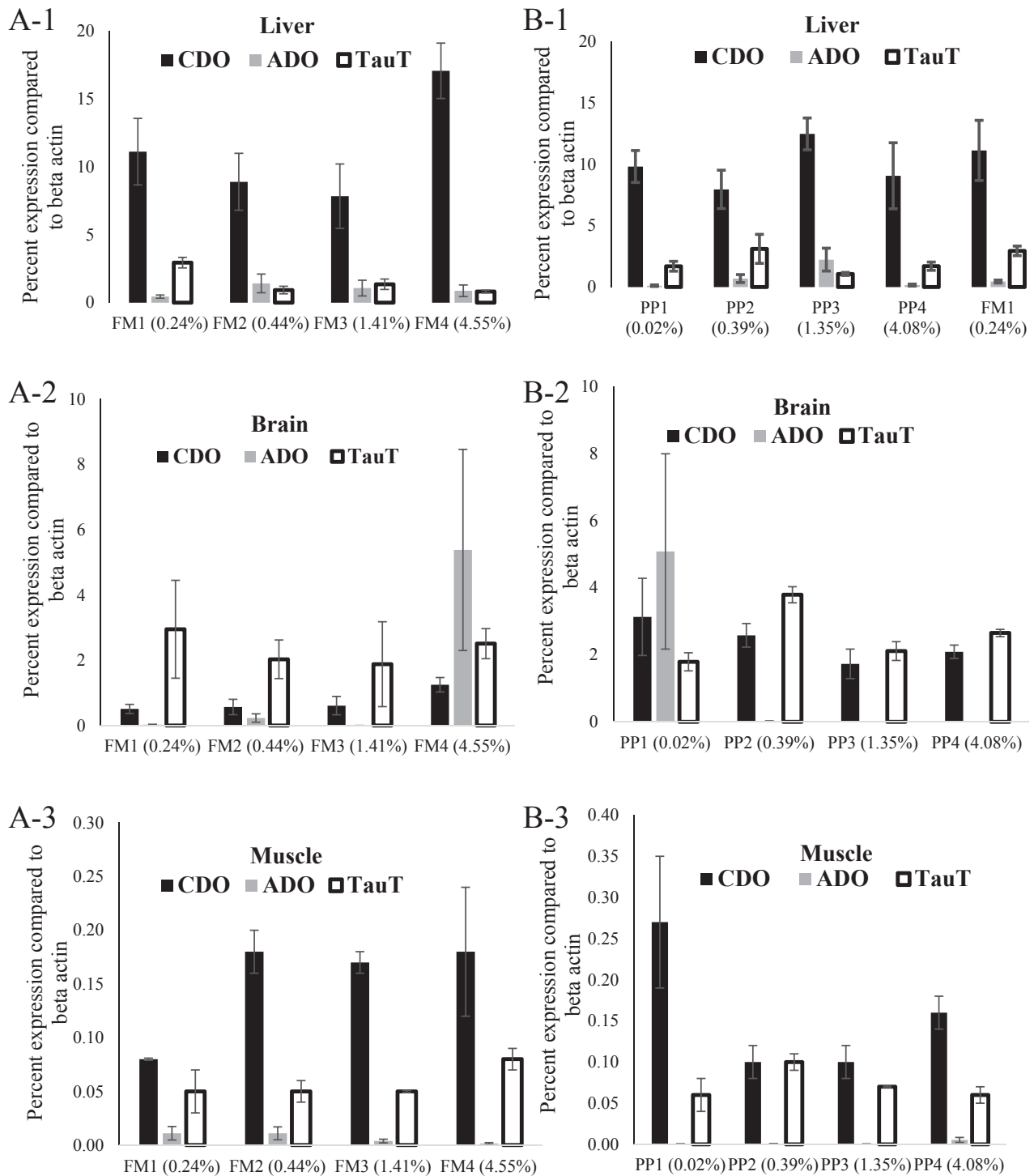
Fig. 1. Relative transcript expression (mean ± SE) of genes involved in taurine synthesis and transport in juvenile cobia, *Rachycentron canadum*, as a percent expression compared to reference gene beta-actin for each diet (% taurine) in liver (1), brain (2), and muscle (3). Equivalent cDNA input (10 ng) for triplicate samples of each tissue and six fish sampled per treatment group and tissue (*n* = 6 per data point). Numbers in parenthesis indicate measured taurine in each diet. Panels A-1, A-2, and A-3 depict results from Watson *et al.* (2014). with fishmeal based diet series and panels B-1, B-2, and B-3 depict results from fishmeal free, plant protein based diet series.

Fillet Fatty Acid Compositions
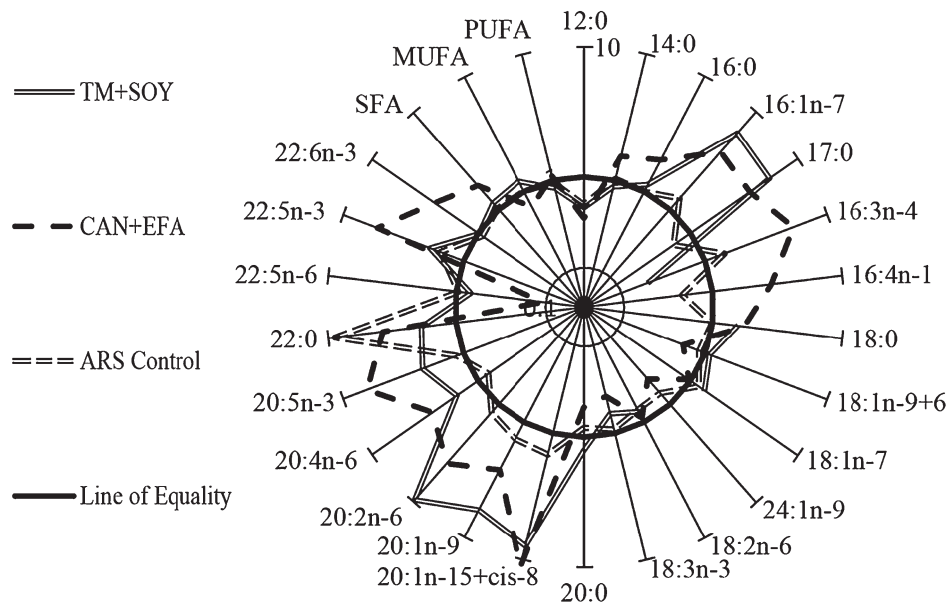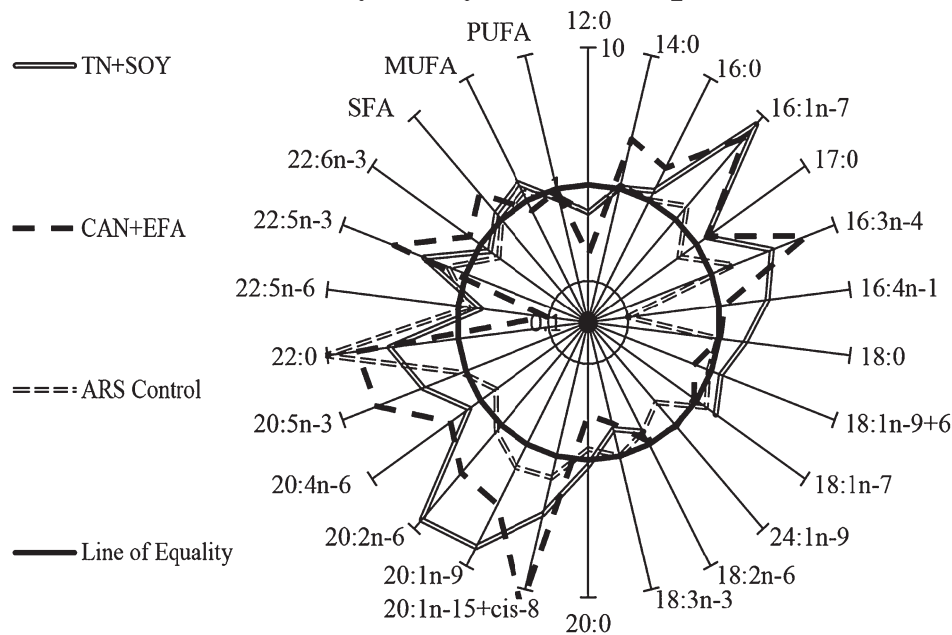

Whole Body Fatty Acid Compositions

Fig. 2. Fatty acid compositions of fillet and whole body tissues from juvenile cobia fed experimental fish oil replacement diets expressed as a fraction of dietary total lipid profile. Values were calculated from relative fatty acid methyl ester (FAME) composition (Fillet or whole body fatty acid concentration/Diet fatty acid concentration). Based on this calculation, a value of 1 represents equality between fillet and dietary fatty acid composition. From Watson *et al.*, 2013a.

source diets also resulted in lower total lipid content of the fillets with the TM+SOY diet resulting in 5.81 ± 0.77% lipid, the CO+EFA diet resulting in 5.53 ± 3.15% lipid, and the control ARS fish oil diet resulting in 7.90 ± 1.75% lipid.

Discussion

These studies illustrate the importance of determining species specific requirement levels for compounds which need to be supplemented separately to feeds. Results from the taurine supplementation to a fishmeal based diet indicate that diets containing at least 35% fishmeal contain sufficient taurine and do not require additional supplementation. However, the trial with a fishmeal free, plant protein based formulation reveal negative effects of low taurine such as low growth and high feed conversion, which are alleviated by increasing dietary taurine, up to ~5.0% supplementation. These effects, combined with the lack of modulation at the transcript level of genes involved in potential taurine synthesis and transport appear to indicate that juvenile cobia are unable to synthesize taurine in sufficient quantities, if at all. Taurine should therefore be considered essential, especially when reducing fishmeal in favor of plant based proteins.

DHA and ARA levels were most likely insufficient in the CO+EFA diet to meet the nutritional requirements of juvenile cobia. This also potentially indicates limited or no synthetic capacity for these specific fatty acids in this species. Gilthead sea bream, *Sparus aurata*, in contrast performed equivalently on all three of the exact same diets from this study (Watson *et al*., 2013b). The difference in performance of these two species on the same diets could highlight the difference in requirement levels for these fatty acids between species, or different synthetic or conversion capabilities between these two carnivores.

Taken together, these studies demonstrate the ability to produce fishmeal and fish oil free diets for a fast growing, high value marine carnivore without significant loss of production characteristics. Targeted supplementation of specific compounds found to be lacking in fishmeal and fish oil replacement sources can alleviate deficiencies. This type of work will allow for the aquaculture industry to continue to expand to meet growing global needs. Species specific work also has the potential to produce optimal diets which not only maximize production but reduce losses due to waste. These two characteristics, high production and low waste, will be critical in expanding aquaculture operations of various scales and intensity while maintaining profitability around the world as global priorities shift to food and water supply safety and the environmental impacts of aquaculture.

References

Arendt M. D., Olney J. E., and Lucy J. A., 2001: Stomach content analysis of cobia, *Rachycentron canadum* from lower Chesapeake Bay. *Fish. Bull*., **99**, 665–670.

Chatzifotis S., Polemitou I., Divanach P., and Antonopoulou E., 2008: Effect of dietary taurine supplementation on growth performance and bile salt activated lipase activity of common dentex, *Dentex dentex*, fed a fish meal/soy protein concentrate-based diet. *Aquacult*., **275**, 201–208.

FAO, 2012: The State of World Fisheries and Aquaculture. food Agric. Organ. United Nations Rome, Italy.

Franks J. S., Garber N. M., and Warren J. R., 1996: Stomach contents of juvenile cobia, *Rachycentron canadum*, from the northern Gulf of Mexico. *Fish. Bull*., **94**, 374–380.

Glencross B., Rutherford N., and Jones B., 2011: Evaluating options for fishmeal replacement in diets for juvenile barramundi (*Lates calcarifer*). *Aquac. Nutr*., **17**, 722–732.

Holt G. J., Faulk C. K., and Schwarz M. H., 2007: A review of the larviculture of cobia *Rachycentron canadum*, a warm water marine fish. *Aquacult*., **268**, 181–187.

Kader M. A., Bulbul M., Koshio S., Ishikawa M., Yokoyama S., Nguyen B. T., and Komilus C. F., 2012: Effect of complete replacement of fishmeal by dehulled soybean meal with crude attractants supplementation in diets for red sea bream, *Pagrus major*. *Aquacult*., **350–353**, 109–116.

Kim S., Takeuchi T., Akimoto A., Furuita H., Yamamoto T., Yokoyama M., and Murata Y., 2005: Effect of taurine supplemented practical diet on growth performance and taurine contents in whole body and tissues of juvenile Japanese flounder *Paralichthys olivaceus*. *Fish. Sci.*, **71**, 627–632.

Kim S., Takeuchi T., Yokoyama M., and Murata Y., 2003: Effect of dietary supplementation with taurine, b-alanine and GABA on the growth of juvenile and fingerling Japanese flounder *Paralichthys olivaceus*. *Fish. Sci.*, **69**, 242–248.

Lunger A. N., Craig S. R., and McLean E., 2006: Replacement of fish meal in cobia (*Rachycentron canadum*) diets using an organically certified protein. *Aquacult.*, **257**, 393–399.

Lunger A. N., McLean E., Gaylord T. G., Kuhn D., and Craig S. R., 2007: Taurine supplementation to alternative dietary proteins used in fish meal replacement enhances growth of juvenile cobia (*Rachycentron canadum*). *Aquacult.*, **271**, 401–410.

Luo L., Xue M., Wu X., Cai X., Cao H., and Liang Y., 2006: Partial or total replacement of fishmeal by solvent-extracted cottonseed meal in diets for juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquac. Nutr.*, **12**, 418–424.

Pinto W., Figueira L., Ribeiro L., Yúfera M., Dinis M. T., and Aragão C., 2010: Dietary taurine supplementation enhances metamorphosis and growth potential of *Solea senegalensis* larvae. *Aquacult.*, **309**, 159–164.

Rust M. B., Barrows F. T., Hardy R. W., Lazur A. L., Naughten K., and Silverstein J., 2011: NOAA/USDA Alternative Feeds Initiative. The Future of Aquafeeds. NOAA Tech. Memo. NMFS–F/SPO–124 103 p.

Salze G., McLean E., Battle P. R., Schwarz M. H., and Craig S. R., 2010: Use of soy protein concentrate and novel ingredients in the total elimination of fish meal and fish oil in diets for juvenile cobia, *Rachycentron canadum*. *Aquacult.*, **298**, 294–299.

Schuller Levis G. B., and Park E., 2003: Taurine: new implications for an old amino acid. *FEMS Microbiol. Lett.*, **226**, 195–202.

Thiessen D. L., Maenz D. D., Newkirk R. W., Classen H. L., and Drew M. D., 2004: Replacement of fishmeal by canola protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss*). *Aquac. Nutr.*, **10**, 379–388.

Trushenski J., Schwarz M., Bergman A., Rombenso A., and Delbos B., 2012: DHA is essential, EPA appears largely expendable, in meeting the n–3 long-chain polyunsaturated fatty acid requirements of juvenile cobia *Rachycentron canadum*. *Aquacult.*, **326–329**, 81–89.

Wacyk J., Powell M., Rodnick K., Overturf K., Hill R. A., and Hardy R., 2012: Dietary protein source significantly alters growth performance, plasma variables and hepatic gene expression in rainbow trout (*Oncorhynchus mykiss*) fed amino acid balanced diets. *Aquacult.*, **356–357**, 223–234.

Watson A. M., Barrows F. T., and Place A. R., 2014: Effects of graded taurine levels on juvenile cobia, *Rachychentron canadum*. *Nor. Am. J. Aquacult.*, **76**, 190–200.

Watson A. M., Barrows F. T., and Place A. R., 2013a: Taurine supplementation of plant derived protein and n-3 fatty acids are critical for optimal growth and development of cobia, *Rachycentron canadum*. *Lipids*, **48:9**, 899–913.

Watson A. M., Barrows F. T., and Place A. R., 2013b: Taurine supplemented plant protein based diets with alternative lipid sources for juvenile gilthead sea bream *Sparus aurata*. *J. Fish. Aquac.*, **4**, 59–66.

Watson A. M., Kissil G. W., Barrows F. T., and Place A. R., 2012: Developing a plant-based diet for cobia *Rachycentron canadum*. *Int. Aquafeed*, **15**, 34–38.

Xie S., He X., and Yang Y., 1998: Effects on growth and feed utilization of Chinese longsnout catfish *Leiocassis longirostris* Günther of replacement of dietary fishmeal by soybean cake. *Aquac. Nutr.*, **4**, 187–192.